DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355-1958
203/572-5200 FAX: 203/572-5240

April 23, 1996

Office of Pesticide Programs - H7505C
Biopesticide and Pollution Prevention Division
U.S. Environmental Protection Division
Document Processing Desk
Room 266A, Crystal Mall #2
1921 Jefferson Davis Highway
Arlington, VA 22202

Attn: Mr. Phil Hutton
Team Leader 90

Dear Mr. Hutton:

Please find enclosed 16 volumes which are submitted in support of a request for registration plant pesticide *Bacillus thruringiensis* subsp. *kurstaki* CryIA(c) protein in corn and exemption from the requirement for tolerances for CryIA(c) and phosphinothricin acetyltransferase proteins. These volumes are submitted by DEKALB Genetics Corporation as the owner and original submitter, and DEKALB retains all rights, title, and interest in this data, including but not limited to exclusive use and data compensation rights.

DEKALB requests that our application for registration and exemptions from the requirement for tolerances be considered in time to market seed in February of 1997. If there are any questions concerning this submission, please do not hesitate to contact me by telephone, (203) 572-5207, or by e-mail, mspencer@dekalb.com.

Sincerely,

T. Michael Spencer
Manager, Regulatory Affairs

cc: Dr. Christopher E. Flick, DEKALB, w/out enclosures
Dr. Catherine J. Mackey, DEKALB, w/out enclosures
Dr. Michael A. Stephens, DEKALB, w/out enclosures
Mr. Richard O. Ryan, DEKALB, w/out enclosures
Dr. James White, USDA/APHIS, w/out enclosures

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Volume 9: Lacetti, L., Adams, W.R., Nutkis, J.E. and Walters, D.S. "Characterization of the Phosphinothricin Acetyltransferase Protein from Transgenic Plants and Demonstration of Equivalence to Microbially Produced Phosphinothricin Acetyltransferase", a study conducted by DEKALB Genetics Corporation. DEKALB Study No. DGC-95-A20

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Volume 10: Stephens, M.A., "Environmental Fate of CryIA(c) Protein", a study conducted by DEKALB Genetics Corporation. DEKALB Study No. DGC-96-A24

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Volume 12: Walters, D.S., and Kruger, D.E., "Evaluation of the Pollen Expression of CryIA(c) Insecticidal Protein in DEKALB Transformant DBT418 Using *Manduca sexta* Toxicity Assays", a study conducted by DEKALB Genetics Corporation. DEKALB Study No. DGC-95-A21

Volume 13: Collins, M. K., "Transgenic Maize Leaf Tissue and Microbially Produced CryIA(c) Protein: Chronic Toxicity to Collembola (*Folsomia candida*)", a conducted by Springborn Laboratories, Inc., under contract to DEKALB Genetics Corporation (Springborn Study No. 13601-1195-6101-123). DEKALB Study No. DGC-95-A16

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Volume 16: Stephens, M.A, and Spencer, T.M., "Insect Resistance Management Plan for Corn Containing *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Protein", a study conducted by DEKALB Genetics Corporation. DEKALB Study No. DGC-96-A25

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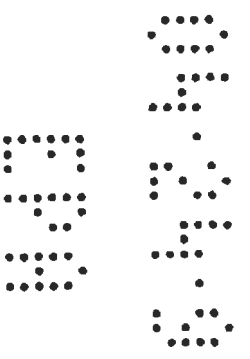
Section I. Administrative Materials



Please read instructions on reverse before completing form. Form Approved. OMB No. 2070-0060. Approval expires 05-31-98

United States
Environmental Protection Agency
Washington, DC 20460

[XXX] Registration
[ ] Amendment
[ ] Other

OPP Identifier Number: 246915

Application for Pesticide - Section I

1. Company/Product Number: DEKALB Genetics Corp./

2. EPA Product Manager: Phil Hutton

3. Proposed Classification: [XX] None [ ] Restricted

4. Company/Product (Name): DEKALB Genetics Corp./Corn Borer - Resistant Corn Containing Insecticidal Bt Protein

PM#: 90

5. Name and Address of Applicant *(Include ZIP Code)*
T. Michael Spencer
DEKALB Genetics Corp.
3100 Sycamore Rd.
DeKalb, IL 60115-9600
[ ] *Check if this is a new address*

6. **Expedited Review.** In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:
EPA Reg. No. _____
Product Name _____

Section - II

[ ] Amendment - Explain below.
[ ] Resubmission in response to Agency letter dated _____
[ ] Notification - Explain below.
[ ] Final printed labels in response to Agency letter dated _____
[ ] "Me Too" Application.
[ ] Other - Explain below.

Explanation: Use additional page(s) if necessary. (For section I and Section II.)

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistant Packaging	Unit Packaging	Water Soluble Packaging	2. Type of Container
[ ] Yes* [X] No	[ ] Yes [X] No	[ ] Yes [X] No	[ ] Metal [ ] Plastic [ ] Glass [ ] Paper [X] Other (Specify) Plant Cells
* *Certification must be submitted*	If "Yes" Unit Packaging wgt. / No. per container	If "Yes" Package wgt / No. per container	

3. Location of Net Contents Information: [ ] Label [ ] Container

4. Size(s) Retail Container: N/A

5. Location of Label Directions: [ ] On Label [ ] On Labeling accompanying product

6. Manner in Which Label is Affixed to Product: [ ] Lithograph [ ] Paper glued [ ] Stenciled [X] Other _____

Section - IV

1. Contact Point *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
T. Michael Spencer	Manager, Regulatory Affairs	(860) 572-5200

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

2. Signature	3. Title	6. Date Application Received (Stamped)
T. Michael Spencer	Manager, Regulatory Affairs	
4. Typed Name: T. Michael Spencer	5. Date: April 23, 1996	

White - EPA File Copy (optional) Yellow - Applicant Copy

EPA

United States
Environmental Protection Agency
Washington, DC 20460

Form Approved
OMB No. 2070-0060
Approval Expires 05-31-95

Certification with Respect to Citation of Data

Applicants Name and Address	EPA File Symbol/Registration Number
T. Michael Spencer DEKALB Genetics Corp. 3100 Sycamore Rd. DeKalb, IL 60115-9600	Product Name: Corn borer - resistant corn containing insecticidal Bt protein Date of Application: April 23, 1996

NOTE: If your product is a 100% repackaging of another EPA-registered product that you purchase, and is labeled for the same uses, you do not need to submit this form. You must submit the Formulator's Exemption Statement (EPA Form 8570-27).

1. This application is supported by all data submitted or cited in the application. In addition, if cite-all options are indicated, this application is supported by all data in the Agency's files that concern the properties or effects of this product that is identical or substantially similar and that is one of the types of data that would be required to be submitted if this application sought the initial registration of a product of identical or similar composition and intended uses under the data requirements in effect on the date of approval of this application. (Check the appropriate boxes, in items 2 and 3, or 4 below that pertain to your application.)

2. I certify that, for each study cited in support of this application for registration that is an exclusive use study.

   [XX] I am the original submitter*; or

   [XX] I have obtained the written permission of the original submitter for CryIA(c) protein (insert name of chemical), which is Monsanto (insert names of companies) (for multiple chemicals link the companies who are original data submitters with the appropriate chemical name) to cite that study*

3. I certify that, for each study cited in support of this application for registration that is not an exclusive use study;

   a. [X] I am the original data submitter*; or

   [ ] I have obtained the written permission of the original data submitter for _____ (insert name of chemical), which is _____ (insert names of companies) (for multiple chemicals link the companies who are original data submitters with the appropriate chemical name) to cite that study*; or

   b. [ ] I have notified in writing the companies _____ (insert names of companies) for _____ (insert name of chemical) that have submitted data I have cited to support this application and have offered to: (a) Pay compensation for those data in accordance with section 3(c)(1)(F) and 3(c)(2)(D) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); and (b) Commence negotiations to determine which data are subject to the compensation requirement of FIFRA and the amount and terms of compensation due, if any. The companies I have notified are:

   Companies _____ (insert names of companies) for _____ (insert name of chemical) (for multiple chemicals link the companies who are original data submitters with the appropriate chemical name) listed on the Pesticide Data Submitters List for all active ingredients contained in my product (cite-all method or cite-all option under Selective Method*). (Also, sign the General Offer Statement below.)

   Companies _____ (insert names of companies) for _____ (insert name of chemical) (for multiple chemicals link the companies who are original data submitters with the appropriate chemical name) that have submitted the studies which I have cited (Selective method*).

4. [ ] I certify that for each study cited in support of this application I am not required to offer data compensation or obtain written permission because all time periods for exclusive use and data compensation have expired.

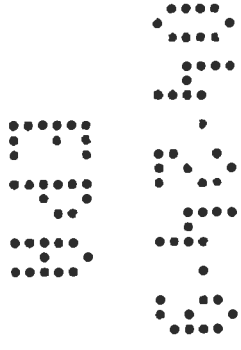
* A Data Matrix identifying these studies is attached. (Note: a Data Matrix is not required under the cite-all method)

Signature	Name and Title	Date
T. Michael Spencer	T. Michael Spencer/Manager, Reg. Affairs	April 23, 1996

General Offer to Pay: I hereby offer and agree to pay compensation to other persons, with regard to the approval of this application, to the extent required.

Signature	Name and Title	Date
T. Michael Spencer	T. Michael Spencer/Manager, Reg. Affairs	April 23, 1996

Section II. Identification of the Data Requirements for the Application - Correspondence Between DEKALB and the EPA



DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355-1958
203/572-5200 FAX: 203/572-5240

Mike Mendelsohn
Biopesticides and Pollution Prevention Division (7501W)
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive (5th Floor)
Arlington, VA 22202

August 4, 1995

Dear Mr. Mendelsohn,

You have previously received a proposal (May 1, 1995) from DEKALB Genetics Corporation outlining the data to be developed from field grown material to support FIFRA registration and tolerance exemptions for the CryIA(c) Bt protein and phosphinothricin acetyltransferase (PAT) protein produced in DEKALB's Bt corn lines. This proposal also contained appendices outlining non-field related data requirements. The proposal was reviewed at a subsequent meeting on May 10, 1995 which was attended by the following individuals:

Representing EPA:	Mike Mendelsohn
	Clay Beegle
	Robert Torla
	Roy Sjoblad
Representing DEKALB:	Mike Stephens
	Don Walters
	Fred Betz (consultant)

Subsequent to the May 10 meeting Mike Stephens had phone conversations with Clay Beegle and Bob Rose to clarify some of the ecotoxicity data requirements. This letter summarizes the conclusions from the May 10 meeting and the subsequent phone conversations, outlines DEKALB schedule for collection and submission of

data to the EPA and proposes data requirements to allow the planting of larger acreage of Bt corn prior to full-registration.

Data collection for full registration and exemption from tolerance for CryIA(c) and PAT proteins.

The field data to be collected was outlined in the proposal (copy attached) that was previously submitted to the EPA and our understanding is that the data, when collected, should be sufficient to determine the general levels of accumulation of the Bt and PAT proteins in the plants and will provide adequate indications of the routes of exposure of the transgenic proteins to humans, animals and the environment.

The regulated transgenic proteins will be the Bt protein, since it is the insecticide, and the PAT protein as an inert substance, since it is produced by the selectable marker gene. For one of DEKALB's Bt corn lines, which contains an intact protease inhibitor (PIN) gene, GLP studies will be carried out on field or greenhouse plants to confirm previous non-GLP studies that no detectable transgenic PIN protein is produced by the plants.

Mammalian toxicology studies will be conducted on Bt and PAT proteins produced in microbes. Studies will be conducted to validate that the microbial proteins are equivalent to the proteins produced in the plants. If a *Bacillus thuringiensis* strain is used as a source of Bt protein, the cells will be grown under non-GLP conditions but the resulting protein will be verified to be comparable to the plant protein under GLP. As described in our May 1 proposal, we will compare the molecular weight (SDS-PAGE), immunogenicity (Western blot), glycosylation and amino-terminal sequences of the plant and microbially produced proteins under GLP conditions.

Provided no transgenic PIN protein is detected in the transgenic plants, no toxicology studies for the PIN protein will be required.

Concerning toxicology and fate studies, based on our May meeting, the data collection remains essentially the same as it was outlined in our May 1, 1995 proposal with minor changes allowing options for the avian testing and removing the need for tests on beneficial predators. In summary the studies would include:

1. **Mammalian toxicity:**
   To demonstrate low mammalian toxicity, DEKALB will sponsor an acute oral toxicity test of Bt and PAT proteins on mice. The proteins will be tested independently at a maximum dose of about 5 grams per kg. The stability of the Bt and PAT proteins in simulated (*in vitro*) gastric and intestinal conditions will be reported. To evaluate potential for allergenicity we will examine the Bt and PAT proteins for glycosylation and similarity to known allergens.

2. **Avian toxicity:**
DEKALB will sponsor a simultaneous toxicity test of Bt and PAT protein on bobwhite quail. Grain or leaf material harvested from the Bt plants, will be tested against quail in a short term dietary study (e.g., 5-day feeding followed by 3-day observation). The plant material will be incorporated into quail diet to provide an adequate food source. Concentrated plant extracts may be added to the meal to ensure that the Bt and PAT protein concentrations will be at least the maximum level found in the plant.

3. **Honey bee larvae:**
If Bt is detected in pollen above the LC_{25} for an insect species normally sensitive to the CryIA(c) protein, we will carry out a toxicity test on honey bee larvae and *Daphnia* (as a representative aquatic invertebrate). If the amount of Bt present in pollen is below the LC_{25} of a Bt-sensitive insect we will request a waiver from conducting honey bee toxicity testing.

4. **Soil invertebrates:**
During our May 10 discussions, EPA personnel indicated that the agency was in the process of considering whether testing against *Collembola* (springtails) and earthworms will be mandatory for registration of Bt plant pesticides. Based on a recent phone discussions with Bob Rose (7-12-95) it appears that testing for toxicity against earthworms and *Collembola* will not be necessary if we can demonstrate that exposure is low. If we can demonstrate that Bt released from senescing corn plants into the top 15 cm of soil does not represent any significant exposure, we will request a waiver from conducting toxicity testing on soil invertebrates. We suggest that exposure would be significant if the concentration of Bt in the soil is high enough to exceed the LC_{25} for an insect sensitive to the Bt protein. The insect used in the test will have a sensitivity to Bt that is medium to high relative to the range of insect species capable of being affected by the Bt protein.

5. **Beneficial invertebrates:**
Other than testing for toxicity to honey bees (see point 4 above) our understanding is that the agency will not require toxicity testing against beneficial invertebrates, such as ladybug beetle or parasitoids.

6. **Fish:**
The EPA indicated that no significant exposure of fish to Bt corn plants or plant parts would be anticipated under a range of growing conditions. Accordingly DEKALB will submit a request for a waiver from conducting toxicity feeding studies against fish.

7. **Environmental fate:**
Environmental fate will be examined by determining the distribution of Bt protein in plant parts. especially post-harvest senescing tissues. For risk assessment purposes it will be assumed that all the Bt protein in post-harvest tissue will be distributed in the first 15 cm of soil. Consideration will also be given to any scientific literature which has reported the stability of Bt proteins in soil. If calculations reveal that the concentration of Bt in the soil will be less than the LC_{25} for an invertebrate that is sensitive to CryIA(c) Bt protein, we suggest that no additional data collection for fate in soil should be necessary and we will request a waiver from conducting further environmental fate studies.

Proposed timetable for full registration of DEKALB's Bt corn.

DEKALB is currently collecting data appropriate for submission to the EPA for registration of our Bt corn product and the issuance of an exemption from tolerance for CryIA(c) Bt protein and PAT protein. It is anticipated that DEKALB will submit these data packages in March - June of 1996 with the main time-limiting step being the generation of data determining the toxicity of the Bt and PAT proteins to mammals and birds. The company is hoping that the agency will be able to review our submission in time to provide approval for the marketing and sale of seed products containing the Bt protein in the spring of 1997. Since optimum sales in the spring of 1997 will require marketing efforts in the winter months, approval as soon as possible in 1997 would reduce the losses the company may incur as a result of competitor Bt corn already approved for sale and marketing.

Proposed timetable and data requirements for larger plantings in 1996.

DEKALB has been testing and collecting data from Bt corn plants in the field since 1993. This testing has been limited to less than 10 acres per insect pest for any calendar year and has been regulated under APHIS regulations. The acreage will also not exceed 10 acres per crop per pest this calendar year. The nature of the seed business is such that significantly more than 10 acres of corn plants will need to be planted in the summer of 1996 to build up inventories of inbred and hybrid seed to allow sale of the Bt corn seed as soon as the product is approved for sale by the EPA, USDA and FDA.

It is anticipated that DEKALB may need to plant 500 acres of corn per transformant source containing the CryIA(c) gene in the late spring (May 15 onwards) of 1996. Since the company may continue to develop two transformant sources with the same Bt gene present, the total acreage for the CryIA(c) gene may be 500-1000 acres,

although it is likely that the area will be at the lower end of this estimate. The purposes for the increase acreage will be:

(a) wide area testing for resistance trait and general product performance,
(b) the generation of grain for quality control testing and
(c) the production of parent inbreds and commercial hybrid seed.

Given the purpose of our 1996 plantings, it is our understanding that a limited registration, rather than an EUP, may be needed. If a limited registration is needed, DEKALB would expect to submit an application in December of 1995. The limited registration submission for product testing and production under biological isolation and crop destruct conditions would include the following information:

(a) A description of the genes introduced into the plants and the methods of introduction used. A description of the encoded proteins, their mode of action and an assessment of their expected toxicity.

(b) A short review of the biology of corn plants (required to assess the containment procedures to be employed see e and f below).

(c) Non-GLP data on the pattern and level of expression of the transgenes introduced into the plants.

(d) Depending on the time required by the EPA to review this information, additional GLP data on gene expression may become available after the submission but before planting.

(e) A description of procedures that will be used to ensure that all plants will be isolated from commercial corn production or breeding plots such that the probability of spread of the genetic information to commercial corn will be negligible.

(f) A description of procedures that will be employed to ensure that no harvested seed or plant material derived from the Bt producing corn plants will enter commerce.

While the initial submission (December, 1995) will not contain actual toxicology data on the safety of the Bt and PAT proteins to mammals or birds, we will make a reasonable case that, based on current knowledge, kurstaki-type Bt proteins are safe to mammals and wildlife and that the PAT protein is also unlikely to be a safety concern. The low probability of exposure, low expected toxicity, and containment procedures to be employed will indicate to the agency that the increased acreage request will have a negligible risk to humans and the environment.

DEKALB would be grateful if the agency would review the summary data of requirements described above and indicate whether we have correctly identified the data and information necessary to allow for:

(i) increased acreage of our Bt corn product in 1996;
(ii) registration of our Bt corn product, and
(iii) given acceptable data, provide a basis for issuance of an exemption from tolerance for the Bt and PAT proteins present in the Bt plants and seed.

Concerning the timetables for limited and full registration, we would also be grateful for input as to whether it is feasible that the agency could review DEKALB submissions within the proposed timetable.

I have recently assumed responsibility for registration of transgenic seed products for DEKALB. I will be working closely with Mike Stephens on registration of our Bt corn lines. Please direct any correspondence relating to registration of DEKALB products to me.

Sincerely,

T. Michael Spencer
Manager, Regulatory Affairs
(203) 572-5207

cc: Chris Flick
Mike Stephens

DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355–1958
203/572–5200 FAX: 203/572–5240

Mike Mendelsohn
Biopesticides and Pollution Prevention Division (7501W)
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive (5th Floor)
Arlington, VA 22202

September 7, 1995

Dear Mr. Mendelsohn:

As we discussed in our telephone conversations of August 30 and this morning, I am writing to propose a time for a conference call to discuss data requirements and timing for DEKALB's planned applications for Limited and Full Registration of our Bt corn lines. I propose that the conference call occur at 9 A.M. on Wednesday, September 20, 1995. Please let me know if this time is convenient for you.

An agenda for the conference call is attached. The agenda raises some new questions and reiterates some of the issues discussed in our August 4th letter to you; I have referenced specific pages of the August 4th letter in the agenda. I also propose to provide you with minutes of the conference call two days following the conversation. Would it be possible for the EPA to provide us with a written response to our August 4th letter and the planned conference call by October 6, 1995?

Sincerely,

T. Michael Spencer
Manager, Regulatory Affairs
(203) 572-5207

cc: Chris Flick
Mike Stephens

Agenda for September 20, 1995 Conference Call With the EPA

I. Limited Registration

A. Is a Limited Registration appropriate for the seed increase and production we wish to perform next spring (August 4th letter, pgs. 4-5)?

B. Is the information we have proposed to include in our Limited Registration application sufficient (August 4th letter, pg. 5)?

C. Is the timing we have proposed for submitting our Limited Registration application (August 4th letter, pg. 5) sufficient to allow for review in time for spring planting (i.e. May, 1996)?

D. How specific do we need to be in the Limited Registration application about locations and acreage?

E. Are there guidelines for the format of a Limited Registration application that differ from those of a Full Registration?

II. Full Registration

A. Do we need to perform a mouse acute oral LD_{50} toxicity test with PAT?

B. Which would be the preferred avian (quail) toxicity study, an acute study (71-1) as performed by Ciba Geigy for Bt corn, or a dietary study (71-2) as performed by Monsanto for Bt cotton and potato?

1. What would be the best form of the protein(s)?

a. Bt-enriched leaf protein (how enriched)?

b. microbially produced proteins(s)?

c. lyophilized leaf?

d. lyophilized leaf plus a microbially produced spike?

C. If we determine that Bt levels in pollen are undetectable or below the LC_{25} of a CryIA(c)-sensitive insect (August 4th letter, pg. 3), do we need to perform:

1. a honey bee toxicity study?

2. an aquatic invertebrate (*Daphnia*) study?

3. a beneficial invertebrate (lady beetle) toxicity study?

4. If PAT is detected in pollen, does that affect the answers to 1, 2, and 3 above?

D. If we can demonstrate that soil invertebrate exposure to Bt is low (i.e. below the LC_{25} of a CryIA(c)-sensitive insect), will we need to perform earthworm and collembola toxicity tests (August 4th letter, pg. 3)?

DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355–1958
203/572–5200 FAX: 203/572–5240

September 21, 1995

Mr. Mike Mendelsohn
Biopesticides and Pollution Prevention Division (7504C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive (5th Floor)
Arlington, VA 22202

Dear Mr. Mendelsohn:

Attached are three copies of the data requirements, as we understand them, for registration and tolerance exemptions for the CryIA(c) and phosphinothricin acetyltransferase (PAT) proteins produced in DEKALB's Bt corn lines. This list of data requirements was compiled from the May 10, 1995 discussion of our initial proposal (May 1, 1995), and the September 19, 1995 conference call that related to our August 4, 1995 and September 7, 1995 letters to you.

We would be grateful if the agency would review the attached list of data requirements and indicate if we have correctly identified the data and information necessary for registration and exemption from tolerance. Thank you for your assistance.

Sincerely yours,

T. Michael Spencer
Manager, Regulatory Affairs

TMS/dkt

Enclosure

cc: Christopher Flick, w/enclosure
Michael Stephens, w/enclosure
Donald Walters, w/enclosure

Data Requirements Proposal for Registration and Tolerance Exemptions for CryIA(c) *Bacillus thuringiensis* Delta Endotoxin and Phosphinothricin Acetyltransferase (PAT) Proteins Produced in DEKALB's Insect Resistant Corn

September 21, 1995

I. Limited Registration

Purpose: For seed increase and hybrid production of Bt corn; this will require 500 - 1,000 acres.

Timing: We plan to submit the application in December 1995. To perform seed increase and hybrid production in the summer of 1996, approval by May 1996 is necessary.

Content: A) Product description (DNA used for transformation, transformation procedure, description of integrated DNA, expression of integrated DNA).

B) Containment (description of procedures to be implemented to insure proper containment of all aspects of transportation, storage, planting, growing, and harvesting Bt corn).

C) Locations and acreage (description of locations by state and county) and acreage of Bt corn to be grown in 1996).

II. Full Registration

Purpose: For registration and tolerance exemptions for CryIA(c) and PAT in transgenic maize.

Timing: We plan to submit in the spring of 1996 (March - June) in order to gain approval in time to market seed for the 1997 growing season (late 1996/early 1997).

Content: A) Product analysis

1) Molecular analysis (transgene content, copy number).

2) Demonstration of Mendelian inheritance and stability.

3) CryIA(c) and PAT levels in field-grown plants; demonstration of lack of PINII and β-lactamase proteins.

4) Equivalence of microbially-produced and plant produced CryIA(c) and PAT.

B) Mammalian toxicology

1) Mouse acute oral LD_{50} using microbially-produced CryIA(c) protein.

2) Mouse acute oral LD_{50} using microbially-produced PAT protein.

3) *In vitro* digestibility of CryIA(c) and PAT proteins.

4) Assessment of the allergenicity of CryIA(c) and PAT proteins.

C) Biological and chemical fate

1) Discussion of the biological fate (weediness, outcrossing ability, probable fate of Bt corn products).

2) Chemical fate (crop residue determination).

D) Ecological effects

1) Avian toxicity study

a) Perform a tobacco hornworm bioassay using transgenic grain: if CryIA(c) in grain is below the LC_{25} for hornworm, we will request a waiver based on lack of exposure; if CryIA(c) is above the LC_{25} for hornworm, we will perform a dietary quail toxicity test using lyophilized leaf tissue.

2) Honey bee, fish, aquatic invertebrate (*Daphnia*), and nontarget beneficial organism toxicity studies

a) Perform a tobacco hornworm bioassay using transgenic pollen: if CryIA(c) in pollen is below the LC_{25} for hornworm, we will request waivers to the studies above based on lack of exposure; if CryIA(c) is above the LC_{25} for hornworm, we will perform the necessary studies.

3) Beneficial terrestrial invertebrates

a) Perform collembola toxicity test using lyophilized leaf plus additional protein (microbially produced or enriched leaf) to obtain approximately the level of Bt protein used by Ciba-Geigy.

b) Perform earthworm toxicity test using same test material used in collembola study above.

TMS/dkt
9/21/95

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 25 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

T. Michael Spencer
Manager, Regulatory Affairs
Dekalb Genetics Corporation
Discovery Research
62 Maritime Drive
Mystic, CT 06355-1958

Dear Mr. Spencer:

Subject: Your Letter Dated 9/21/95 Regarding Data Requirements for Bt Corn

The Biopesticides and Pollution Prevention Division has reviewed your letter and has determined that it accurately reflects our position with the following exceptions.

1) In the full registration section under product analysis item 3, you indicate that you will demonstrate the lack of certain proteins in field grown plants. If you choose to do this, you must provide your detection limit. An alternate approach acceptable to the Agency would be to provide scientific rationale as to why these proteins would not be expressed, i.e. they are not under a eukaryotic promoter, etc.

2) In the full registration section under mammalian toxicology items 1 and 2, you may devise/propose a limit dose study rather than full LD_{50} studies.

Sincerely,

Janet L. Andersen
Acting Director
Biopesticides and Pollution
Prevention Division (7501W)

DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355-1958
203/572-5200 FAX: 203/572-5240

February 21, 1996

Mr. Michael Mendelsohn
Biopesticide and Pollution Prevention Division (7501W)
Environmental Protection Agency
2800 Crystal Drive (5th Floor)
Arlington, VA 22202

Dear Mr. Mendelsohn:

DEKALB Genetics Corporation and Monsanto Company have recently entered into a collaborative research and development agreement. As part of this agreement, DEKALB has received permission from Monsanto to reference data that was submitted to the EPA in support of Monsanto's existing plant pesticide registrations. As you are aware, DEKALB is in the process of applying for registration and tolerance exemptions for transgenic field corn containing *Bacillus thuringiensis* CryIA(c) protein. We plan to submit our application for full registration and tolerance exemptions for this product in March, 1996.

This newly formed research and development collaboration provides a unique opportunity for reducing the redundancy of data submitted to EPA for registration of similar products. In the process of registering our CryIA(c) corn, DEKALB proposes to reference data submitted by Monsanto for registration of Bollgard™ Cotton containing CryIA(c) protein. Applicable data includes acute toxicity, in vitro digestion, non-target insect, and environmental fate data. If this data is bridged to the DEKALB application it may significantly reduce the amount of data for review by the EPA and facilitate approval of our product.

Attached is an outline of the proposed DEKALB submission and reference to the Monsanto CryIA(c) data that may bridge to our package. Also enclosed is background information demonstrating the equivalence of the Monsanto and DEKALB CryIA(c) proteins. It is possible for DEKALB to submit all of our available data in addition to citing applicable Monsanto data, however we would like an opinion from the EPA on what alternative would be the most beneficial-to-all approach.

DEKALB and Monsanto would like to consult with the EPA on this matter. We would like to meet with you and the appropriate reviewers in person, at your earliest convenience, to

discuss the possibility of bridging Monsanto data to the DEKALB application. We propose March 7, 8, 12, or 13 for the meeting.

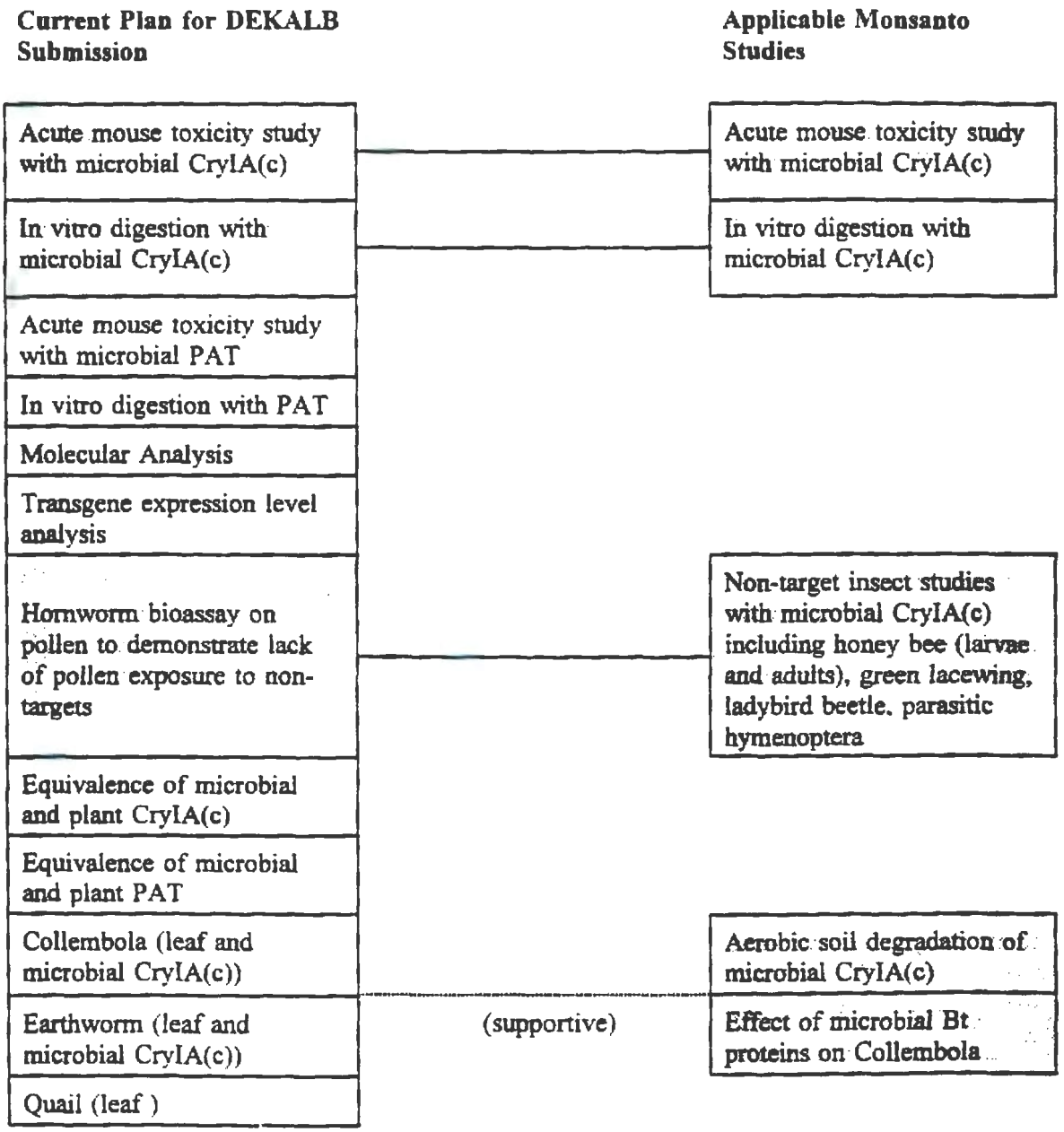
Sincerely,

T. Michael Spencer
Manager, Regulatory Affairs
(203) 572-5207

Enclosure

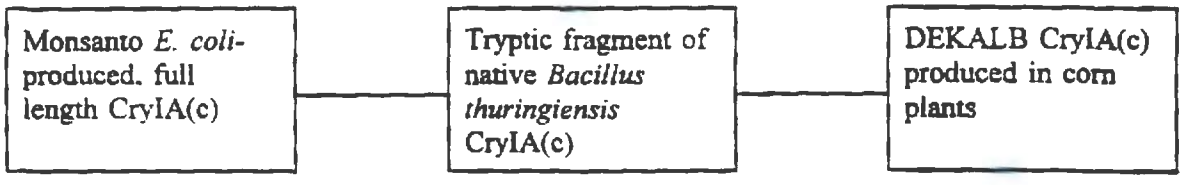
cc: Christopher Flick, DEKALB w/attachment
Michael Stephens, DEKALB w/attachment
Donald Walters, DEKALB w/attachment
Kent Croon, Monsanto w/attachment
Roy Fuchs, Monsanto w/attachment
Russ Schneider, Monsanto w/attachment
Phil Hutton, EPA w/attachment
Fred Betz, JSC w/attachment

Proposed Bridging of Monsanto CryIA(c) Studies to DEKALB CryIA(c) Corn application



Equivalence of Monsanto and DEKALB CryIA(c) Proteins

The diagram below schematically depicts the proposed bridge between the Monsanto Bollgard™ cotton data and the DEKALB CryIA(c) corn registration application.



The CryIA(c) protein used by Monsanto as the test substance in toxicity studies to support Bollgard™ cotton registration was a full length CryIA(c) protein produced in *E. coli* (Sammons, 1994, MRID No. 4314503). The insecticidally active, trypsin-resistant core of the Monsanto CryIA(c) protein is 99% identical (differing by six out of about 600 amino acids) to the trypsin-resistant core of the native *Bacillus thuringiensis* CryIA(c) protein. The Monsanto, full length, *E. coli*-produced CryIA(c) protein was demonstrated to be functionally equivalent to the tryptic fragment of native *B. thuringiensis* CryIA(c) when tested in bioassays against ten insect species representing five different orders (Sims, 1994, MRID No. 43145204).

The CryIA(c) protein produced in DEKALB's corn lines is identical to the first 613 amino acids of the native *B. thuringiensis* CryIA(c) protein and includes the insecticidally active, trypsin resistant core. DEKALB plans to demonstrate the physical and functional equivalence of the CryIA(c) protein produced in transgenic corn to the tryptic core of the native *B. thuringiensis* CryIA(c) protein as outlined below:

- molecular weight estimation by SDS-PAGE
- immunoreactivity as determined by Western blot
- insecticidal activity

In summary, the amino acid sequences of the tryptic fragments of the Monsanto and DEKALB CryIA(c) proteins have greater than 99% homology. The Monsanto CryIA(c) protein is functionally equivalent to the tryptic core of the native *B. thuringiensis* CryIA(c) protein. The CryIA(c) protein in DEKALB's corn lines is functionally equivalent to, and physically indistinguishable from the first 613 amino acids of the native *B. thuringiensis* CryIA(c) protein.

DEKALB Genetics Corporation
DISCOVERY RESEARCH, 62 MARITIME DRIVE, MYSTIC, CT 06355-1958
203/572-5200 FAX: 203/572-5240

Mr. Mike Mendelsohn
Biopesticide and Pollution Prevention Division (7501W)
Environmental Protection Agency
2800 Crystal Drive (5th Floor)
Arlington, VA 22202

April 2, 1996

Dear Mr. Mendelsohn:

On March 12, 1996, representatives from DEKALB Genetics Corporation, Monsanto Company, and the EPA met to discuss a data-bridging proposal submitted by DEKALB to the EPA in a letter dated February 26, 1996. In this proposal, DEKALB requested to reference data previously submitted to the EPA by Monsanto, to support DEKALB's registration and tolerance exemption application for corn containing *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) protein. DEKALB intends to submit this application to the EPA later this month. The purpose of this letter is to summarize the discussions and conclusions of the March 12 meeting.

The purpose of the March 12 meeting was to determine if studies submitted in support of Monsanto's CryIA(c) cotton registration were applicable to DEKALB's CryIA(c) corn registration application. Present at the meeting were myself, representing DEKALB and Kent Croon representing Monsanto. Representing the EPA were Mike Mendelsohn, Bob Rose, Roy Sjoblad, Cindy Schaffer, Janet Anderson, and Phil Hutton.

DEKALB initiated the meeting by summarizing the February 26 letter from DEKALB to the EPA. In this letter, the similarity of the CryIA(c) proteins in Monsanto's cotton and DEKALB's corn was described. DEKALB proposed that Monsanto's acute mouse toxicity, *in vitro* digestion, non-target insect, and soil degradation studies, that were submitted in support of CryIA(c) cotton registration, were appropriate to reference in support of DEKALB's application.

The EPA indicated that the Monsanto CryIA(c) mouse toxicity and *in vitro* digestion studies were appropriate to support DEKALB's CryIA(c) corn registration, however, because the agency is aware that DEKALB has generated similar data, DEKALB must submit that data. It was agreed that DEKALB could cite the Monsanto mouse toxicity and *in vitro* digestion data to strengthen the application.

Regarding Monsanto's CryIA(c) non-target insect studies, the EPA requested that DEKALB submit the completed DEKALB study involving CryIA(c) pollen fed to tobacco hornworms to demonstrate lack of exposure to honey bees. The EPA stated that the Monsanto lady beetle, parasitic wasp, and green lacewing CryIA(c) toxicity studies could be referenced by DEKALB to demonstrate lack of toxicity to non-target insects.

The EPA stated that the aerobic soil degradation study submitted by Monsanto could be referenced as supportive, but would not be a sufficient substitute for collembola and earthworm studies. DEKALB agreed to submit earthworm and collembola studies.

Several other issues related to DEKALB's CryIA(c) corn registration application, but unrelated to the data-bridging proposal were discussed. The EPA stated that DEKALB should address potential exposure fish to CryIA(c) protein via fish food made with corn grain. DEKALB agreed to investigate the effect of fish food processing on the insecticidal activity of CryIA(c) in grain. The EPA emphasized the importance of an insect resistance management (IRM) plan and stated the DEKALB should submit an IRM plan with the registration package for the package to be complete. DEKALB noted its concern about the IRM issue and agreed to submit a proposal with the registration package. DEKALB asked if a complete registration package is submitted to the agency in April of 1996, is approval in time to market seed for the 1997 growing season possible? The agency responded that "anything is possible", but approval by January 1997 is unlikely.

DEKALB's limited registration application for seed increase and production in 1996 was also discussed. The EPA expressed concern about DEKALB's request to plant over 5000 acres of CryIA(c) corn in 1996. DEKALB stated that the acreage actually needed is closer to 2000 acres. The EPA agreed to discuss the issue further.

This letter is intended to summarize and document the discussions at the March 12 meeting. Please feel free to contact me if you have any questions or comments concerning this summary.

Sincerely,

T. Michael Spencer
Manager, Regulatory Affairs

cc: Chris Flick, DEKALB
Mike Stephens, DEKALB
Kent Croon, Monsanto
Fred Betz, JSC

Section III. Permission from Monsanto to Reference Studies Previously Submitted to the EPA

Monsanto

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone (314) 694-1000

April 9, 1996

Dekalb Genetics Corporation
Discovery Drive
62 Maritime Drive
Mystic, CT 06355-1958

Attn: Michael Spencer
Dekalb Genetics Corp.

RE: Citation of Monsanto Studies in support of Dekalb Petition for Registration for the *B. thuringiensis* subsp. *kurstaki* delta endotoxin protein expressed in corn by the CryIA(c) gene.

Dear Mr. Spencer:

Monsanto submits this letter to you as our notice regarding the citation of pesticide product studies currently at the EPA. These studies were submitted by Monsanto company as the owner and original submitter of data in support of Pesticide Petition 4F4331 for the registration of the CryIA(c) protein as expressed in cotton and Pesticide Petition 5F4473 for the registration of the CryIA(b) proetin as expressed in corn. It is our understanding that Dekalb Genetics Corp. has requested to cite this information in support of a submission to the agency for the CryIA(c) protein as expressed in corn. Monsanto retains ownership and rights in that data, including but not limited to exclusive use and data compensation rights.

Studies submitted by Monsanto:

EPA MRID 43145203	Sammons, R. D. "Characterization of Purified *B.t.k.* HD-73 Protein Produced in *Escherichia coli*" (1994), Study Number 92-01-36-18, an unpublished study conducted by Monsanto Company.
EPA MRID 43145212	Sammons, R.D. "*B.t.k.* HD-73 Protein Dose Formulation and Determination of Dose for An Acute Mouse Feeding Study MD 92-493" (1994), Study Number 92-01-36-13, an unpublished study by Monsanto.
EPA MRID 43145213	Naylor, M.W. "Acute Oral Toxicity of *Bacillus thuringiensis* var. *kurstaki* [Cry IA(c)] HD-73 protein in Albino Mice" (1993), Study Number ML-92-493, an unpublished study by Monsanto.

EPA MRID 43145214	Ream, J.E. "Assessment of the In vitro Digestive Fate of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein"(1994), Laboratory Project ID Number 92-01-36-22, an unpublished study conducted by Monsanto.
EPA MRID 43145204	Sims, S.R. "Sensitivity of Insect Species to the Purified CryIA(c) Insecticidal Protein from *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.* HD-73)" (1994), Study Number 92-01-36-17, an unpublished study conducted by Monsanto Company.
EPA MRID 43145205	Sims, S.R. "Stability of the CryIA(c) Insecticidal Protein of *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.* HD-73) in Sucrose and Honey Solutions Under Non-refrigerated Temperature Conditions" (1994), Study Number 92-01-36-15, an unpublished study conducted by Monsanto Company.
EPA MRID 43145206	Maggi, V.L. "Evaluation of the Dietary Effects(s) of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Larvae" (1993), Study Number 92-01-36-10, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto Company.
EPA MRID 43145207	Maggi, V.L. "Evaluation of the Dietary Effect(s) of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Adults" (1993), Study Number 92-01-36-10, an unpublished study conducted by California Agricultural Research, Inc. under contract to Monsanto Company.
EPA MRID 43145208	Palmer, S.J. and Beavers, J.B. "*B.t.k.* HD-73 Protein: A dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*)" (1993), Study Number WL-93-234, an unpublished study conducted by Wildlife International Ltd. under contract to Monsanto.
EPA MRID 43145209	Palmer, S.J. and Beavers, J.B. "*B.t.k.* HD-73 Protein: A dietary Toxicity Study with Ladybird Beetles (*Hippodamia convergens*)" (1993), Study Number WL-93-232, an unpublished study conducted by Wildlife International Ltd. under contract to Monsanto.
EPA MRID 43145210	Palmer, S.J. and Beavers, J.B. "*B.t.k.* HD-73 Protein: A dietary Toxicity Study with Green Lacewing Larvae (*Chrysopa carnea*)" (1993), Study Number WL-93-233, an unpublished study conducted by Wildlife International Ltd. under contract to Monsanto.

EPA MRID 43145211	Cambell, S.M. and Beavers, J.B., "A Dietary Toxicity Study with Cotton Seed Meal in the Northern Bobwhite" (1993), Study Number WL-93-13, an unpublished study conducted by Wildlife International, Ltd. under contract to Monsanto.
EPA MRID 43145215	Ream, J.E. "Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein Bioactivity" (1994), Study Number 92-01-36-21, an unpublished study conducted by Monsanto Company.
EPA MRID 43533206	Sims, S.R., and P.R. Sanders. "Aerobic Soil Degradation of Bacillus thuuringiensis var. Kurstaki HD-1 Protein" (1994), Study Number 94-01-39-13, an unpublished study conducted by Monsanto Company.
EPA MRID 43941601	Sims, S.R. And Martin, J.W. "Effect of the *Bacillus thuringiensis* insecticidal proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola)" (1996), an unpublished study conducted by Monsanto Company.
EPA MRID 43887901	Jackson, L.S., E.H. Robinson, D.L. Nida, and P.R. Sanders. "Evaluation of the European Corn Borer Resistant Corn Line MON 801 as a Feed Ingredient for Catfish" (1994), Study Number 94-01-39-16, an unpublished study conducted by Monsanto Company.

Monsanto understands that Dekalb will offer to pay compensation to the extent required by FIFRA as amended, with respect to citation of that data to support and approval of the petition, under Section 3(c)(1)(F) of the Act and 40 CFR 152.93.

Sincerely,

Kent A. Croon, Ph.D.
Regulatory Affairs Manager

Section IV. Summary of the Application

Summary of the Application

A. Introduction

DEKALB Genetics Corporation has developed transgenic corn lines (*Zea mays* L.) that are resistant to attack by European corn borer larvae due to the introduction and expression of a *Bacillus thuringiensis* subsp. *kurstaki cryIA(c)* gene. Field testing of these plants under USDA/APHIS notification in 1993, 1994 and 1995 has demonstrated extremely good resistance to European corn borer, an important insect pest of corn in the U.S. DEKALB believes that providing corn growers in the U.S. with plants highly resistant to European corn borers will contribute to increased corn yields with decreased use of less selective chemical insecticides. The result will be reduced costs, increased profitability for the farmer and, by reducing the use of chemical insecticides, an improvement in the quality of the environment. Summarized below is data that is being submitted in support of registration of a transgenic corn line designated DBT418.

B. Product Analysis

1. **Introduction of DNA.** Plasmid DNA was introduced into embryogenic corn cells using microprojectile bombardment (Stephens and Walters, 1995, MRID No. 43871401). The cell culture was initiated from immature embryos of an inbred line designated AT824. Transgenic callus was selected for its ability to grow on medium containing herbicide (see below). Plants were regenerated from transgenic callus by placing the callus on media which stimulate the production of shoots and roots. Regenerated plants were crossed with non-transgenic, inbred corn lines to produce seed that inherited the inserted transgenes. Repeated backcrossing to various inbred lines was performed to create inbred hybrid germplasm containing the DBT418 insertion event.

2. **DNA used for transformation.** The insect resistance gene that was used to produce transgenic corn line DBT418 encodes a protein that is identical to the first 613 amino acids of a naturally occurring *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) protein. The *cryIA(c)* gene in the corn line DBT418 consists of a DNA sequence that has been modified to contain an increased number of codons that are preferred for expression in maize (Stephens and Walters, 1995, MRID No. 43871401). The CryIA(c) protein that is produced, however, is still identical to the first 613 amino acids residues of the naturally occurring CryIA(c) protein.

Also used to produce transgenic corn line DBT418 was the herbicide resistance gene *bar* (Stephens and Walters, 1995, MRID No. 43871401). The *bar* gene was originally isolated from *Streptomyces hygroscopicus* and encodes phosphinothricin acetyltransferase (PAT). This enzyme is useful as a selectable marker as well as a source of resistance to the herbicide phosphinothricin, also known as glufosinate, the active ingredient in several herbicide formulations manufactured by AgrEvo.

In DNA preparations used to produce DBT418, a plasmid containing the protease inhibitor II gene from potato (*pinII*) was also included (Stephens and Walters, 1995, MRID No. 43871401). If produced at sufficient concentrations in plants, certain protease inhibitors have been shown to have insecticidal activity against specific insects. A single incomplete and nonfunctional copy of the *pinII* gene was shown to be present in DBT418 (see below).

3. **Molecular characterization of DBT418 plants.** DBT418 plants were characterized as to copy number and stability of the introduced DNA (Volume 5). Southern blot analysis indicated that DBT418 plants contained a single insertion that consisted of approximately two copies each of *cryIA(c)* and *bar*, and a single, incomplete copy of *pinII*. Also included in the DBT418 insertion were multiple copies of the *E. coli* antibiotic resistance marker gene *bla*, which encodes β-lactamase, and the ColE1 origin of replication. The DBT418 insertion event was demonstrated to be stable at the molecular level (Volume 5) and was shown to be inherited in a Mendelian fashion (Volume 6).

4. **Transgene expression analysis of DBT418 plants.** DBT418 plants were analyzed for CryIA(c), PAT, pinII, and β-lactamase protein expression levels (Volume 7). CryIA(c) protein was detected at maximum levels of 1198, 124, 125, 43, 111, and 147 ng/g dry weight in leaf, stalk, root, kernel, silk, and whole plants, respectively. No CryIA(c) was detected in DBT418 pollen. PAT protein was detected at maximum levels of 1099, 136, 95, 6, 133, and 120 µg/g dry weight in leaf, stalk, root, kernel, silk, and whole plants, respectively. No PAT was detected in DBT418 pollen. As expected given the lack of an intact *pinII* gene, no pinII protein was detected in DBT418 plants. Lack of expression of the β-lactamase gene was also demonstrated.

5. **Equivalence of microbially-produced and plant produced CryIA(c) and PAT.** CryIA(c) and PAT proteins were purified from microbial sources to provide adequate amounts of the proteins for toxicity testing. The microbially produced proteins were characterized and compared to plant produced proteins to demonstrate equivalence (Volumes 8 and 9). Microbially produced CryIA(c) and PAT were compared to their plant produced counterparts in

terms of their molecular weight, immunoreactivity, glycosylation, amino-terminal sequence, and activity. Microbially produced CryIA(c) and PAT were shown to be suitable surrogates for the corresponding plant produced proteins.

C. Human Health Effects

1. **CryIA(c) mouse acute oral gavage.** Mice were administered a dose of 3325 mg CryIA(c)/kg body weight by oral gavage (Volume 2). No acute toxicity was observed. The oral LD_{50} for CryIA(c) in mice determined from this study is greater than 3325 mg/kg. This finding is further supported by a similar study performed by Monsanto (MRID No. 43145213). In this study, the LD_{50} for CryIA(c) in mice was found to exceed 4300 mg/kg.

2. **PAT mouse acute oral gavage.** Mice were administered a dose of 2500 mg PAT/kg body weight by oral gavage (Volume 3). No acute toxicity was observed. The oral LD_{50} for PAT in mice determined from this study is greater than 2500 mg/kg.

3. **Digestibility of CryIA(c) and PAT.** As part of the mammalian safety assessment, CryIA(c) and PAT proteins were tested for digestibility using simulated gastric fluid (Volume 4). Both proteins were found to rapidly degrade in full strength and dilute simulated gastric fluid. CryIA(c) degraded to below detection limits after a few seconds in full strength (1X) simulated gastric fluid. In simulated gastric fluid in which the pepsin concentration had been reduced 100-fold (0.01X), CryIA(c) degraded to below detection in five minutes. PAT degraded to trace levels after 2 minutes in full strength (1X) simulated gastric fluid. No PAT was detectable after 5 minutes in 0.01X simulated gastric fluid. Degradation of CryIA(c) in simulated gastric fluid is further supported by data generated by Monsanto (MRID No. 43145214). This study demonstrated rapid loss of immunologically detectable CryIA(c) and CryIA(c) bioactivity following exposure to simulated digestive fluid. The rapid degradation of CryIA(c) and PAT proteins supports the safety of these proteins for mammalian consumption.

D. Ecological Effects

1. **Dietary toxicity study with quail.** The purpose of this study was to assess the potential toxicity of corn containing CryIA(c) protein to quail (Volume 11). An avian toxicity test was performed given the potential exposure of birds to corn grain containing CryIA(c) protein. The test was performed

using young quail and lyophilized DBT418 leaf tissue incorporated at 20% w/w (200,000 ppm) into diet. Leaf tissue was chosen as the test substance due to the fact that CryIA(c) protein levels were highest in DBT418 leaves of any DBT418 tissues tested (Volume 7). No mortalities or overt signs of toxicity were observed for any of the test or control birds. All birds were normal in appearance and behavior throughout the test period. No treatment-related effects on body weight or feed consumption were observed. The no observed effect level for lyophilized DBT418 leaf was considered to be greater than 20% w/w.

2. **Tobacco Hornworm bioassay of DBT418 pollen to demonstrate lack of pollen-based exposure to non-target organisms.** The level of CryIA(c) protein in DBT418 pollen was evaluated using a tobacco hornworm, THW (Volume 12). THW was chosen as a test organism given that it is extremely sensitive to CryIA(c) protein. The assays were performed by incorporating fresh or freeze-dried pollen into an agarose matrix. The pollen and agarose served as the only food source for THW for a period of five days. No toxic effects, mortality or growth inhibition were observed over multiple repetitions of the five-day assay. These data indicate that CryIA(c) expression in DBT418 pollen is so low as to present no risk of toxicity to non-lepidopterous insect species such as the honeybee or other non-target insects or to fish that may be exposed to DBT418 pollen.

3. **Monsanto studies on the lack of effect of CryIA(c) on honey bee larvae and adults, lady beetle, green lacewing, and parasitic wasp.** CryIA(c) protein was tested for toxicity to honey bee larvae and adults (MRID Nos. 43145206 and 43145207). Honey bee larvae and adults were exposed to 0.2 ppm CryIA(c) protein; no toxicity was observed. Lady beetles were exposed to 20 ppm CryIA(c) protein in diet for 30 days, no toxicity was observed (MRID No. 43145209). Green lacewing larvae were exposed to 20 ppm CryIA(c) protein over a period of 11 days, no toxicity was observed (MRID No. 43145210). Parasitic Hymenoptera were exposed to 20 ppm CryIA(c) protein over a period of 23 days, no toxicity was observed (MRID No. 43145208). These results demonstrated the lack of toxicity of CryIA(c) protein to a range of non-target insects.

4. **Chronic toxicity study with collembola.** The purpose of this study was to determine the chronic toxicity of CryIA(c) protein in DBT418 plants to collembola. A collembola toxicity test was performed given the potential exposure to collembola via incorporation of DBT418 plant material into the soil (Volume 13). Two test substances were chosen for the test: lyophilized DBT418 leaf tissue and microbially-produced CryIA(c) protein. Leaf tissue was chosen as a test substance to most accurately represent exposure to

collembola in the field. Two concentrations of lyophilized leaf were tested: 2 and 8 g/kg soil. Microbially-produced CryIA(c) was also used for the test to provide exposure levels that greatly exceed typical exposure in the field. CryIA(c) was tested at 0.1 mg/kg soil. Survival of the test organisms was reduced in the test substance and control substance treatments, as compared to the untreated control. However, no difference in survival was observed between test substance treatments and the corresponding control substance treatments. No differences between offspring production were observed between the test substances, control substances, or the untreated controls.

5. **Acute toxicity study with earthworms.** The purpose of this study was to determine the acute toxicity of CryIA(c) protein in DBT418 plants to earthworms. An earthworm toxicity test was performed given the potential exposure of earthworms to CryIA(c) protein via incorporation of DBT418 plant material into the soil (Volume 14). As in the collembola study described above, two test substances were chosen for the test: lyophilized DBT418 leaf tissue and microbially-produced CryIA(c) protein. Test substance treatments consisted of two different concentrations of DBT418 leaf tissue (2 and 8 g/kg soil) and one combination of DBT418 leaf tissue and microbially-produced CryIA(c) protein (2 g leaf + 0.1 mg CryIA(c)/kg soil). No effect on earthworm mortality was observed at any of the test substance concentrations. No difference in weight gain was observed between test substance treatments and corresponding control treatments.

6. **Demonstration of lack of exposure to fish.** DBT418 corn grain was processed into catfish food using an extrusion procedure that is typically used for making fish foods. The resulting catfish food was incorporated into European corn borer diet to test for insecticidal activity (Volume 15). No insecticidal activity was detected, demonstrating lack of CryIA(c) exposure to farm-raised fish that would potentially be fed food made from DBT418 grain.

E. Environmental Fate

The amount of CryIA(c) protein per acre of DBT418 corn was calculated to be approximately 1.0 g (Volume 10). This value represents the maximal amount of CryIA(c) that would be incorporated into the soil following harvest. Reports in the scientific literature and studies submitted by Monsanto (MRID Nos. 43145211 and 43533206) demonstrate rapid loss of insect activity following incorporation of *Bacillus thuringiensis* subsp. *kurstaki* proteins into soil. Earthworm (Volume 14 and collembola (Volume 13) studies described above demonstrated no adverse effects following incorporation of DBT418 leaf or purified CryIA(c) into the soil.

F. Insect Resistance Management

DEKALB is committed to the implementation of an effective resistance management strategy (Volume 16). DEKALB recognizes the potential risk for the development of insects that are resistant to *Bacillus thuringiensis* subsp. *kurstaki* proteins and is committed to practices that will reduce that risk while maintaining the availability of this important product to growers.

Section V. Proposed Labeling

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

Section VI. Data Reference List

Data Reference List

Studies Submitted or Referenced in Support of Registration and Tolerance Exemptions	MRID Number	submission date	document number	owner
Product Analysis				
Summary of Product Analysis for *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Protein Produced in Corn Plants	43871401	11/30/95	none	DEKALB
Molecular Characterization of Transgene Content and Stability in Transgenic Corn Hybrid Line DK.DL (DBT418)		4/23/96	DGC-95-A07	DEKALB
Demonstration of Stable Mendelian Inheritance of *cryIA(c)* and *bar* Genes in DBT418		4/23/96	DGC-95-A14	DEKALB
Magnitude of Transgenic Protein Accumulation in Transformed DBT418 Corn Lines		4/23/96	DGC-95-A01	DEKALB
Characterization of the CryIA(c) Protein from Transgenic Plants and Demonstration of Equivalence to Microbially Produced CryIA(c)		4/23/96	DGC-95-A19	DEKALB

Studies Submitted or Referenced in Support of Registration and Tolerance Exemptions	MRID Number	submission date	document number	owner
Product Analysis (continued)				
Characterization of the Phosphinothricin Acetyltransferase Protein from Transgenic Plants and Demonstration of Equivalence to Microbially Produced Phosphinothricin Acetyltransferase		4/23/96	DGC-95-A20	DEKALB
Toxicology				
An Acute Oral Toxicity Study in Mice with *Bacillus Thuringiensis* subsp. *kurstaki* CryIA(c) Delta Endotoxin		4/23/96	DGC-95-A17	DEKALB
An Acute Oral Toxicity Study in Mice with Phosphinothricin Acetyltransferase (PAT) Protein		4/23/96	DGC-95-A18	DEKALB
In vitro Digestibility of CryIA(c) and PAT Proteins		4/23/96	DGC-95-A22	DEKALB
Acute Oral Toxicity of *Bacillus thuringiensis* var. *kurstaki* [CryIA(c)] HD-73 protein in Albino Mice	43145213	2/15/94	MI.-92-493	Monsanto
Assessment of the *in vitro* Digestive Fate of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein	4314214	2/15/94	92-01-36-22	Monsanto

Studies Submitted or Referenced in Support of Registration and Tolerance Exemptions	MRID Number	submission date	document number	owner
Environmental Fate				
Environmental Fate of CryIA(c) Protein		4/23/96	DGC-95-A24	DEKALB
Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein Bioactivity	43145215	2/15/94	92-01-36021.	Monsanto
Ecological Effects				
Lyophilized DBT418 Leaf Tissue: A Dietary Toxicity Study with the Northern Bobwhite		4/23/96	DGC-95-A13	DEKALB
Evaluation of the Pollen Expression of CryIA(c) Insecticidal Protein in DEKALB Transformant DBT418 Using *Manduca sexta* Toxicity Assays		4/19/96	DGC-95-A22	DEKALB
Transgenic Maize Leaf Tissue and Microbially Produced CryIA(c) Protein: Chronic Toxicity to Collembola (*Folsomia candida*)		4/23/96	DGC-95-A16	DEKALB
Lyophilized DBT418 Maize Leaf Tissue and Microbially Produced CryIA(c) Protein - Acute (14-day) Toxicity to Earthworms (*Eisenia foetida*)		4/23/96	DGC-95-A15	DEKALB

Studies Submitted or Referenced in Support of Registration and Tolerance Exemptions	MRID Number	submission date	document number	owner
Ecological Effects (continued)				
Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Larvae	43145206	2/15/94.	92-01-36-10/92-427-709	Monsanto
Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Adults	43145207	2/15/94	92-01-36-10/92-427-708	Monsanto
B.t.k. HD-73 Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitrpennis*)	43145208	2/15/94	WL-93-234	Monsanto
B.t.k. HD-73 Protein: A Dietary Toxicity Study with Ladybird Beetles (*Hippodamia convergens*)	43145209	2/15/94	WL-93-232	Monsanto
B.t.k. HD-73 Protein: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysopa carnea*)	43145210	2/15/94	WL-93-233	Monsanto
Evaluation of CryIA(c) Activity in DBT418 Grain Following Processing into Fish Food		4/23/96	DGC-95-A16	DEKALB

Studies Submitted or Referenced in Support of Registration and Tolerance Exemptions	MRID Number	submission date	document number	owner
Insect Resistance Management				
Insect Resistance Management Plan for Corn Containing *Bacillus thuringiensis* subsp. *kurstaki* CrylA(c) Protein		4/23/96	DGC-96-A25	DEKALB

Section VII. Tolerance Exemption Petitions

Requests for Tolerance Exemptions

Petitions for exemptions from the requirement of a tolerance for:

A.) *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) protein and the genetic material necessary for the production of this protein in or on all raw agricultural commodities when used as a plant-pesticide active ingredient

and

B.) Phosphinothricin acetyltransferase protein and the genetic material necessary for the production of this protein in or on all raw agricultural commodities when used as a plant-pesticide inert ingredient

were filed concurrently with this application for pesticide registration and are supported by data submitted with this registration application.

Form Approved. OMB No. 2070-0060. Approval expires 9-30-90.

EPA

ENVIRONMENTAL PROTECTION AGENCY
REGISTRATION DIVISION (TS-767)
WASHINGTON, D.C. 20460

DATA REFERENCE SHEET

(See Instructions on the back of the last page before completing.)

1. PAGE: 1 OF 3

2. EPA REGISTRATION NO. / FILE SYMBOL: 6937-5

3. APPLICANT'S NAME AND ADDRESS
DEKALB Genetics Corporation
3100 Sycamore Rd.
DeKalb, IL 60115-9600

4. PRODUCT NAME: **Corn Borer-Resistant Corn Containing Insecticidal Bt Protein**

5. PRODUCT MANAGER: **Phil Hutton/90**

6. TO ACCOMPANY APPLICATION FOR REGISTRATION DATED: **April 23, 1996**

7. NAME OF STUDY	SOURCE OF STUDY					8. ACCESSION NUMBER (if known)
	a. APPLICANT CONDUCTED STUDY (mark 'X')	b. OBTAINED FROM EPA (mark 'X')	c. OBTAINED FROM ANOTHER FIRM OR SOURCE (give name and address)	d. OBTAINED FROM PUBLIC LITERATURE (give reference)	e. OTHER (explain)	
Summary of Product Analysis for *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Protein Produced in Corn Plants	X					43871401
Vol. 1: Administrative Materials in Supporte of the Registration of the Plant Pesticide *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Insect Control Protein and Exemption from the Requirement of a Tolerance for CryIA(c) and Phosphinothricin Acetyltransferase Proteins	X					
Vol. 2: An Acute Oral Tocicity Study in Mice with *Bacillus thuringiensis* subsp. *kurstaki* CryI(c) Delta Endotoxin	X					
Vol. 3: An Acute Oral Tocicity Study in Mice with Phosphinothricin Acetyltransferase (PAT) Protein	X					
Vol. 4: *In vitro* Digestibility of CryIA(c) and PAT proteins	X					
Vol. 5: Molecular Characterization of Transgene Content and Stability in Transgenic Corn Hybrid Line DK.DL(DBT418)	X					
Vol. 6: Demonstration of Stable Mendelian Inheritance of *cryIA(c)* and *bar* genes in DBT418	X					
Vol. 7: Magnitude of Transgenic Protein in Transformed DBT418 Corn Lines	X					
Vol. 8: Characterization of the CryIA(c) Protein from Transgenic Plants and Demonstration of Equivalence to Microbially Produced CryIA(c)	X					

If you marked "X" in this column, do you wish your name placed on the Data Submitters List? ☒ YES ☐ NO

Form Approved. OMB No. 2070-0060. Approval expires 9-30-90.

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ENVIRONMENTAL PROTECTION AGENCY
REGISTRATION DIVISION (TS-767)
WASHINGTON, D.C. 20460

DATA REFERENCE SHEET

(See Instructions on the back of the last page before completing.)

1 PAGE: 2 OF 3

2. EPA REGISTRATION NO. / FILE SYMBOL: 69573-F

3. APPLICANT'S NAME AND ADDRESS
DEKALB Genetics Corporation
3100 Sycamore Rd.
DeKalb, IL 60115-9600

4 PRODUCT NAME: **Corn Borer-Resistant Corn Containing Insecticidal Bt Protein**

5. PRODUCT MANAGER: **Phil Hutton/90**

6. TO ACCOMPANY APPLICATION FOR REGISTRATION DATED: **April 23, 1996**

7. NAME OF STUDY	SOURCE OF STUDY a. APPLICANT CONDUCTED STUDY (mark 'X')	b. OBTAINED FROM EPA (mark 'X')	c. OBTAINED FROM ANOTHER FIRM OR SOURCE (give name and address)	d. OBTAINED FROM PUBLIC LITERATURE (give reference)	e. OTHER (explain)	f. ACCESSION NUMBER (if known)
Vol. 9: Characterization of the Phosphinothricin Acetyltransferase Protein from Transgenic Plants and Demonstration of equivalence to Microbially Produced Phosphinothricin Acetyltransferase	X					
Vol. 10: Environmental Fate of CryIA(c) Protein	X					
Vol. 11: Lyophilized DBT418 Leaf Tissue: A Dietary Toxicity Study with the Northern Bobwhite	X					
Vol. 12: Evaluation of the Pollen Expression of CryIA(c) Insecticidal Protein in DEKALB Transformant DBT418 using *Manduca sexta* Toxicity assays	X					
Vol. 13: Transgenic Maize Leaf Tissue and Microbially Produced CryIA(c) Protein: Chronic Toxicity to Collembola (*Folsmia candida*)	X					
Vol. 14: Lyophilized DBT418 Maize Leaf Tissue and Microbially Produced CryIA(c) Protein - Acute Toxicity to Earthworms (*Eisenia foetida*)	X					
Vol. 15: Evaluation of CryIA(c) Activity in DBT418 Grain Following Processing into Fish Food	X					
Vol. 16: Insect Resistance Management Plan for Corn Containing *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Protein	X					
Acute Oral Toxicity of *Bacillus thuringiensis* var. *kurstaki* (CryIA(c)) HD-73 Protein in Albino Mice			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43145213

If you marked "X" in this column, do you wish your name placed on the Data Submitters List? ☒ YES ☐ NO

Form Approved. OMB No. 2070-0060. Approval expires 9-30-90.

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ENVIRONMENTAL PROTECTION AGENCY
REGISTRATION DIVISION (TS-767)
WASHINGTON, D.C. 20460

DATA REFERENCE SHEET

(See Instructions on the back of the last page before completing.)

1. PAGE: 3 OF 3

2. EPA REGISTRATION NO. / FILE SYMBOL: 69575

3. APPLICANTS NAME AND ADDRESS
DEKALB Genetics Corporation
3100 Sycamore Rd.
DeKalb, IL 60115-9600

4. PRODUCT NAME: **Corn Borer-Resistant Corn Containing Insecticidal Bt Protein**

5. PRODUCT MANAGER: **Phil Hutton/90**

6. TO ACCOMPANY APPLICATION FOR REGISTRATION DATED: **April 23, 1996**

7. NAME OF STUDY	SOURCE OF STUDY a. APPLICANT CONDUCTED STUDY (mark 'X')	b. OBTAINED FROM EPA (mark 'X')	c. OBTAINED FROM ANOTHER FIRM OR SOURCE (give name and address)	d. OBTAINED FROM PUBLIC LITERATURE (give reference)	e. OTHER (explain)	f. ACCESSION NUMBER (if known)
Assessment of the *In vitro* Digestive Fate of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43151452
Aerobic Soil Degradation of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein Bioactivity			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43151455
Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Larvae			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43152066
Evaluation of the Dietary Effects of Purified *B.t.k.* Endotoxin Proteins on Honey Bee Adults			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43152067
B.t.k. HD-73 Protein: A Dietary Study with Parasitic Hymenoptera (*Nasonia vitrpennis*)			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43152068
B.t.k. HD-73 Protein: A Dietary Study with Ladybird Beetles (*Hipodamia convergens*)			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43152069
B.t.k. HD-73 Protein: A Dietary Study with Green Lacewing Larvae (*Chrysopa carnea*)			Monsanto Agricultural Co. 700 Chesterfield Parkway North Chesterfield, MO 63198			43151450

If you marked "X" in this column, do you wish your name placed on the Data Submitters List? ☐ YES ☐ NO

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600

PLANT PESTICIDE ACTIVE INGREDIENT

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein.

Active Ingredient:

Bacillus thuringiensis subsp. *kurstaki* CryIA(c) insect control protein as produced in corn by a *cryIA(c)* gene and its controlling sequences.....100%

DEKALB Genetics Corporation
3100 Sycamore Road
DEKALB, Illinois 60115-9600